Extended Abstract

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Expression of a Shiga-Like Toxin during Plastic Colonization by Two Multidrug-Resistant Bacteria, Aeromonas hydrophila RIT668 and Citrobacter freundii RIT669, Isolated from Endangered Turtles (Clemmys guttata)

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Abstract: Aeromonas hydrophila RIT668 and Citrobacter freundii RIT669 were isolated from endangered spotted turtles (Clemmys guttata). Whole-genome sequencing, annotation and phylogenetic analyses of the genomes revealed that the closest relative of RIT668 is A. hydrophila ATCC 7966 and Citrobacter portucalensis A60 for RIT669. Resistome analysis showed that A. hydrophila and C. freundii harbor six and 19 different antibiotic resistance genes, respectively. Both bacteria colonize polyethylene and polypropylene, which are common plastics, found in the environment and are used to fabricate medical devices. The expression of six biofilm-related genes—biofilm peroxide resistance protein (bsmA), biofilm formation regulatory protein subunit R (bssR), biofilm formation regulatory protein subunit S (bssS), biofilm formation regulator (hmsP), toxin-antitoxin biofilm protein (tabA) and transcriptional activator of curli operon (csgD)—and two virulence factors—Vi antigen-related gene (viaB) and Shiga-like toxin (slt-II)—was investigated by RT-PCR. A. hydrophila displayed a > 2-fold increase in slt-II expression in cells adhering to both polymers, C. freundii adhering on polyethylene displayed a > 2-fold, and on polypropylene a > 6- fold upregulation of slt-II. Thus, the two new isolates are potential pathogens owing to their drug resistance, surface colonization and upregulation of a slt-II-type diarrheal toxin on polymer surfaces.

Keywords: Citrobacter; Aeromonas; biofilm; turtle; Shiga-like toxin; antibiotic resistance; plastic; whole-genome sequencing

Introduction

Antibiotic resistance is an increasing crisis as both the range of resistance in clinical settings expands and the pipeline for development of new antibiotics contracts [1]. This problem is compounded by the global genomic scope of the antibiotic resistome, so that antibiotic resistance spans a continuum from genes in clinical pathogens to those of benign environmental microbes along with their proto-resistance gene progenitors [2,3]. Further, increased resistance to antimicrobial agents occurs in biofilms [4]. Biofilm-associated cells differ from their suspended counterparts by generation of an extracellular polymeric substance (EPS) matrix, reduced growth rates, and the up/downregulation of specific genes [5]. Plasmid conjugation occurs at a greater rate between cells in biofilms than between planktonic cells [6–8]. Bacterial biofilms constitute a serious problem for public health due to their potential to colonize in-dwelling medical devices (IMDs) [9,10], such as abdominal [11] and coronary stents [12], which contain polymeric materials such as polyethylene (PE) and polypropylene (PP). Once infected, the IMDs are often removed and replaced, causing a significant increase in the health care cost and chance of reinfection [13]. Similarly, water supplies contaminated with biofilms are a significant risk for public health.

Materials and Methods

Microbial samples were isolated from 12 adult rescued infected spotted turtles (Clemmys guttata) seized by the United States Fish and Wildlife Service from an illegal reptile trade operation (chain of custody ID number-ST#032797). The spotted turtle is a small, semi-aquatic, North American species commonly targeted and illegally harvested for sale in the pet trade and overseas for other uses [99,100]. The eyes, nostrils and limbs of turtles were swabbed on to agar plates and the samples were initially subjected to biochemical assays. For subsequent analysis, the two strains identified as Aeromonas hydrophila RIT 668 and Citrobacter freundii RIT 669 (based on 16S rDNA sequences) were routinely cultured on blood agar plates (5% sheep blood) and MacConkey plates respectively (BD BBL™, prepared media, 100 mm × 15 mm, San Diego, CA, USA). Hemolysis on the blood plates was examined by observing the presence of complete lysis around the colonies and a clearing on the medium.

Results

When the research was begun, there was 50% mortality, in spite of following appropriate animal care. By the end of the study period, however, all twelve turtles died (mortality rate 100%). Microbial isolates from rescued spotted turtles (Clemmys guttata) were identified as A. hydrophila and C. freundii, and were Gram-negative, beta hemolytic, lactose fermenting, and potential opportunistic pathogens. The two strains were initially identified through the 16S rDNA sequence coupled with NCBI-BLAST searches. Owing to their possible pathogenicity, the genomes were sequenced and annotated; a summary of the genome characteristics is shown in Table 1.

Discussion

Genome analysis of the ATCC 7966-type strain (which is closely related to RIT668) showed it to be metabolically versatile with significant virulence potential and a predicted ability to infect a variety of hosts [134]. Due to the high similarity in the genomes (99%), it is possible that A. hydrophila RIT668 shares this potential for broad metabolic capability, virulence and the ability to infect multiple hosts, including humans. The closest relative of RIT669 is a C. portucalensis strain; C. portucalensis strains can be multidrug resistant and some may be highly resistant livestock-origin pathogens or “superbugs” [135,136]. This raises the possibility that RIT669 may also be multidrug resistant.

Conclusions

The isolated strains were shown to have significant resistomes, with A. hydrophila containing predicted resistance genes to six antibiotic classes and C. freundii containing resistance genes for 19 classes. The expression of many of the genes examined did not follow a specific pattern, since the adhesion to the polymers could be controlled via the complex interplay of several genes that were or were not included in this study. However, the clear exception is slt-II, whose expression is increased in response to either PE or PP for both bacteria. The toxin expression notwithstanding, electron microscopy showed that the adherent cells form structures different from well-studied biofilms growing on media with blood/bile components.

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